Comm.
Dr. Andervont
Dr. Bing
Dr. Huebner

CARCINOGENESIS

#802R2
#802R1 - 7/1/72-6/30/73
#802 -7/1/71 -6/30/72

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

RECEIVED
JAN 31 1973

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☐ Second Renewal ☒

Date: January 30, 1973

1. Principal Investigator (give title and degrees):

Attallah Kappas, M.D. - Professor & Senior Physician

Alvito P. Alvares, Ph.D. - Assistant Professor

2. Institution & address:

The Rockefeller University
66th Street & York Avenue
New York, N. Y. 10021

3. Department(s) where research will be done or collaboration provided:

Laboratory of Metabolism and Pharmacology
Rockefeller University Hospital

4. Short title of study:

Tobacco Smoke: Effects on detoxification mechanisms

5. Proposed renewal date: July 1, 1973 - June 30, 1974

6. How results to date have changed earlier specific research aims:

There has been no basic change with respect to the research aims outlined in the prior progress report. The only additional work planned relates to the study of the effects of coal tar therapy in psoriatic individuals. In addition, the delayed delivery of the Walton Smoke Machine has necessarily led to a deferral of certain animal studies; these are now in progress and we plan to complete them in the coming year.

7. How results to date have changed earlier working hypothesis:

There has been no change in our original working hypothesis.

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Source: https://www.industrydocuments.ucsf.edu/docs/xzyf0090

8. Any additional facilities now required? Describe briefly:

None required.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

None made. The budget for FY 1973-74 reflects anticipated salary increases for Dr. Alvares and his technical assistant.

10. Append outline of experimental protocol for ensuing year. See appendix.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

1. W. Levin, A. H. Conney, A. P. Alvares, I. Merkatz and A. Kappas: Induction of benzo[α]pyrene hydroxylase in human skin. Science 176: 419-420, 1972.

2. A. P. Alvares, A. Kappas, W. Levin and A. H. Conney: Inducibility of benzo[α]pyrene hydroxylase in human skin by polycyclic hydrocarbons. Clinical Pharmacology and Therapeutics, In press, 1973.

3. A. P. Alvares, S. Leigh, A. Kappas, W. Levin and A. H. Conney: Induction of aryl hydrocarbon hydroxylase in human skin. Proceedings of the Second International Symposium on Microsomes and Drug Oxidations to be published in Drug Metabolism and Disposition, In press, 1973.

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12. Summary progress report (append in standard form as separate document, unless recently submitted). See appendix.

Source: https://www.industrydocuments.ucsf.edu/docs/xzyl0000

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Dr. A. Kappas	25%	-----
Dr. A. P. Alvares	100%	$20,000.
Technical		
1 Technical Assistant	100%	10,000.
	Sub-Total for A	30,000.

B. Consumable supplies (by major categories)	
Chemicals and isotopes	3,500.
Laboratory animals	2,500.
Sub-Total for B	6,000.

C. Other expenses (itemize)	
Travel	500.
Preparation of figures, xerox, reprints, publication costs.	1,500.
Upkeep of animal facility	1,500.
Sub-Total for C	3,500.
Running Total of A + B + C	$39,500.

D. Permanent equipment (itemize)

Aminco-Chance Split Beam dual wavelength Spectrophotometer (Final installment attributable to CTR grant #802).

Sub-Total for D	4,500.
E. Indirect costs (15% of A+B+C)	5,925.
Total request	$49,925.

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Source: https://www.industrydocuments.ucsf.edu/docs/kzyl0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. Entire salary of Dr. A. Kappas, secretary and office expenses provided for by Rockefeller University.			
2. Effects of lead poisoning on hepatic heme synthesis (ES-00621) - Dr. A. Kappas, Principal Investigator.	ES-00621	$59,684.	1973-74
3. General research support.	Hoffmann-LaRoche, Inc.	$15,000.	1973-74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. We plan to submit a renewal application for NIH grant ES-00621 in the autumn of 1973.	NIH		

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to:

e Rockefeller University

Mailing address for check:

Miss O. Margery Pedersen, Chief Accountant
The Rockefeller University, New York, N. Y. 10021.

Principal investigator

Typed Name Dr. Attallah Kappas

Signature [signature] Date 1/30/73

Telephone 212 (Area Code) 360-1286 (Number) (Extension)

Responsible officer of institution

Typed Name Dr. Frederick Seitz

Title President

Signature F. Seitz Date 1/30/73

Telephone 212 (Area Code) 360 1234 (Number) (Extension)

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Source: https://www.industrydocuments.ucsf.edu/docs/xzyl0000

APPENDIX: PROGRESS REPORT AND INVESTIGATIONS PLANNED FOR 1973-74

Tobacco smoke: Effects on detoxification mechanisms

1. Induction of aryl hydrocarbon hydroxylase in human skin:

The aryl hydrocarbon hydroxylase enzyme system which hydroxylates polycyclic hydrocarbons has previously been shown to be present in human liver and placenta. In studies completed to date, we have utilized the tissue culture system and grown human skin in presence and in absence of the polycyclic hydrocarbon, benz[a]anthracene. We have established for the first time the presence of aryl hydroxylase in human skin. We have also studied the properties of this enzyme and results show that its properties in skin and liver are similar. The skin hydroxylase, like the hepatic hydroxylase, has an absolute requirement for NADPH and molecular oxygen and the hydroxylation reaction has a pH optimum of 7.4. The microsomal hemoprotein, cytochrome P-450 was also shown to be a key component of the skin hydroxylase system. When skins were incubated in a growth medium containing benz[a]anthracene, 2- to 5-fold increases in the amount of benzo[a]pyrene hydroxylase were observed. In skins obtained from 13 individuals, significant variations in individual inducing capacities were observed. We have postulated that these differences in the degree of induction might be due to genetic differences and that individuals with a lower capacity to metabolize carcinogens may be more susceptible to the carcinogenic action of polycyclic hydrocarbons ingested orally, through inhaled smoke or through skin absorption. Previous studies have demonstrated individual differences in the ability of humans to metabolize several drugs.

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Source: https://www.industrydocuments.ucsf.edu/docs/xzyl0000

A comparison of the hydroxylase activities in human, rat and mouse skins was also carried out. The basal levels of the enzyme in man are lower than in the mouse or rat and the inducibility of the enzyme is greater in human skin than in the mouse or rat skins. A time-course study showed that the maximum induction of benzo[a]pyrene hydroxylase in skin, incubated in the presence of benz[a]anthracene, occurred between 16 to 24 hours.

Human skin is one of the largest organs of the body. The skin of an average man has been estimated to weigh about 3 times as much as his liver, the principal detoxifying organ in the body. The presence of hydroxylase activity and the sheer size of the skin pool suggests a significant role of skin in detoxification processes in man. Since skin biopsy is relatively easy to perform, biopsy samples may be of predictive value in determining the carcinogenic hazard in people exposed to polycyclic hydrocarbons. The results of the above studies have been the subject of 3 publications, which are cited at the end of this report.

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2. Studies on patients treated with coal tar:

Psoriasis is a chronic skin disease characterized by scaling erythematous plaques which may involve single body areas on the entire integuement. The disease is not curable but one of the most effective and widely used therapeutic modalities is based on a regimen described by Goeckerman in 1925. Crude coal tar in a 5 to 10% concentration in petrolatum is applied to all the skin lesions and left in place for 24 hours. The material is then removed with mineral oil and the entire body exposed to ultraviolet radiation sufficient to produce a moderate erythema. This procedure is repeated daily until the lesions have cleared, generally in 2 to 3 weeks. Since crude

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coal tar is a mixture of a number of aromatic hydrocarbons, including benzo[a]pyrene, we have initiated studies designed to assess the effect of skin application of crude coal tar on drug metabolism in man and on benzo[a]pyrene hydroxylase activity in skin biopsy samples. To study drug metabolism, antipyrine and phenacetin, two widely used antiinflammatory and antipyretic agents have been selected for study. Phenacetin and antipyrine were chosen for these studies since smoking has been shown to lower plasma concentrations of phenacetin and since inducers of drug metabolism cause a decrease in antipyrine half-lives. The plasma half-lives of these two drugs are determined prior to and following treatment of psoriatic patients with coal tar, thus each subject serving as his own control. These studies are being presently carried out in collaboration with Dr. David Bickers, a resident dermatologist at the Skin and Cancer Unit, Department of Dermatology, New York University School of Medicine.

3. Effects of cigarette smoking on the disposition of drugs in man:

Studies have been carried out to determine the effects of smoking on the metabolism of drugs in man. Phenylbutazone and antipyrine have been used as model drugs metabolized primarily by the hepatic microsomal mixed function oxidase system. Following administration of these drugs, the disappearance rates of these drugs from plasma (half-life) provides a measure of the functional capacity of the microsomal enzyme system that metabolizes drugs, other foreign compounds and normal body constituents. In studies completed to date in 20 normal healthy volunteers, smokers showed no significant difference in the metabolism of these model drugs when compared with the half-lives obtained in non-smokers.

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During the course of these studies in adults, we also investigated the half-lives of these drugs in children. Contrary to data obtained with animals where the rates of drug metabolism are much slower in young animals in comparison with adult animals, young children showed a significantly faster rate of drug disposition when compared to adults when the same dosage of antipyrine or phenylbutazone was administered on a per kilogram basis. Thus, for example, the mean half-life of antipyrine in 10 children was 6.6 hours compared with a mean half-life of 13.6 hours obtained in 10 adults. This finding is of major therapeutic significance and is the subject of a manuscript being prepared for publication.

4. Studies on cytochrome P-450 and P-448:

Inducers of hepatic drug-metabolizing enzymes have been categorized into two general groups of chemicals. One group of inducers is mainly drugs, for example phenobarbital, dilantin, etc. A second group of inducers comprise the polycyclic hydrocarbons, such as benzo[a]pyrene and 3-methylcholanthrene. A major difference between these two classes of inducers is their effect on cytochrome P-450. Phenobarbital is a potent inducer of cytochrome P-450; in contrast, polycyclic hydrocarbons induce the formation of a spectrally and catalytically different hemoprotein, cytochrome P-448. Studies on these two cytochromes have been carried out in the laboratory in attempts to isolate and purify these microsomal hemoproteins. To date we have achieved a 6-fold purification of these cytochromes. We have also in the course of our studies, discovered the presence of a new form of cytochrome P-448 which though

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spectrally similar to the P-448 induced by polycyclic hydrocarbons differs in catalytic properties from the hemoprotein induced by these hydrocarbons. This new P-448 is induced by a hitherto unknown class of inducers, the polychlorinated biphenyls (PCB's). The PCB's which are newly recognized as environmental pollutants have recently been discovered to be among the most ubiquitous chemical contaminants of animal and human tissues. In excess of 50% of humans tested have residues of these compounds in their adipose tissues. The sources of this contamination are not clear. The striking differences in the enzymatic properties of cytochrome P-448 induced by polychlorinated biphenyls, of cytochrome P-448 induced by polycyclic hydrocarbons, and of cytochrome P-450 induced by drugs is the subject of a manuscript recently submitted for publication in the Proceedings of the National Academy of Sciences. This study is the first demonstration of a class of chemical substances which are structurally different from the polycyclic hydrocarbons and which induce cytochrome P-448.

5. Enzyme induction following exposure to cigarette smoke using the Walton Smoke Machine:

In August, 1972, we received the Smoke Exposure Machine from the Council of Tobacco Research, U.S.A. Studies are now in progress on the effects of cigarette smoke from the University of Kentucky Reference Cigarettes on the induction of aryl hydrocarbon hydroxylase in rat lung, liver, skin and intestines. Rats are conditioned for a week in the machine prior to exposure to cigarette smoke. Varying times of exposure, acute as well as long-term chronic studies are being carried out. Following exposure, livers are removed and drug-metabolizing enzyme activities,

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cytochrome P-450 levels and NADPH-cytochrome c reductase activities are determined. In other tissues, only benzpyrene hydroxylase activities and cytochrome P-450 levels are determined because of the sensitivities of these assay systems. We plan to carry out similar studies, in the coming year, using pregnant rats and studying these effects on the maternal host as well as on the fetuses.

Presentations (February 1, 1972 - January 31, 1973)

1. Induction of aryl hydrocarbon hydroxylase in human skin by A. P. Alvares at the Second International Symposium on Microsomes and Drug Oxidations, Stanford University, Stanford, Calif.; July 31, 1972.
2. The induction of a carcinogen-metabolizing enzyme in human skin by A. Kappas at the Annual meeting of the American Clinical and Climatological Association, Ponte Vedra Beach, Florida; October 31, 1972.
3. Alteration in hepatic microsomal hemoproteins induced by polycyclic hydrocarbons and polychlorinated biphenyls by A. P. Alvares at the Department of Pharmacology, School of Medicine, New York University, New York, N. Y.; January 12, 1973.

Publications (February 1, 1972 - January 31, 1973)

1. W. Levin, A. H. Conney, A. P. Alvares, I. Merkatz and A. Kappas: Induction of benzo[α]pyrene hydroxylase in human skin. Science 176: 419-420, 1972.

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Source: https://www.industrydocuments.ucsf.edu/docs/azy2000

2. A. P. Alvares, A. Kappas, W. Levin and A. H. Conney: Inducibility of benzo[α]pyrene hydroxylase in human skin by polycyclic hydrocarbons. Clinical Pharmacology and Therapeutics, In press, 1973.

3. A. P. Alvares, S. Leigh, A. Kappas, W. Levin and A. H. Conney: Induction of aryl hydrocarbon hydroxylase in human skin. Proceedings of the Second International Symposium on Microsomes and Drug Oxidations to be published in Drug Metabolism and Disposition, In press, 1973.

4. A. P. Alvares, D. R. Bickers and A. Kappas: Polychlorinated biphenyls: A new type of inducer of cytochrome P-448 in the liver. Proceedings of the National Academy of Sciences, submitted for publication, 1973.

Manuscript in Preparation

1. A. P. Alvares S. Kapelner, D. R. Bickers and A. Kappas: Age differences in the rates of drug metabolism in man: Studies with antipyrine and phenylbutazone.

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